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(54) Title: **USE OF SINGLE-CELL PROTEIN AS FEED FOR FISH AND SHELLFISH**

(57) Abstract: The invention relates to a feed material for fish and shellfish, for example a feed material for molluscs such as mussels. In particular, the invention relates to the use of single-cell protein materials and their derivatives (especially homogenized and/or autolysed derivatives) as a feed for fish and shellfish. A preferred single-cell material for use in the invention is that derived from a microbial culture comprising *Methylococcus capsulatus* (Bath) (strain NCIMB 11132), *Ralstonia sp.* DB3 (strain NCIMB 13287) and *Brevibacillus agri* DB5 (strain NCIMB 13289), optionally in combination with *Aneurinibacillus sp.* DB4 (strain NCIMB 13288).

USE OF SINGLE-CELL PROTEIN AS FEED FOR FISH AND SHELLFISH

5 The present invention relates to a feed material
for fish and shellfish, for example a feed material for
molluscs such as mussels. In particular, the invention
relates to the use of single-cell protein materials and
their derivatives as a feed for fish and shellfish.

10 Single-cell microorganisms such as fungi, yeasts
and bacteria have been proposed as new sources of
protein in human foods and as certain animal feeds, e.g.
feeds for chickens, pigs, etc. These may be grown by
single-cell reproduction and several bio-synthetic
15 processes for the production of protein through the
growth of single-cell microorganisms on hydrocarbon and
other substrates have been developed. For example,
PCT/GB01/00628 (published as WO 01/60974), DK 1404/92,
EP-A-418187 and EP-A-306466 describe various methods and
apparatus suitable for the production of such materials.
20 The entire contents of each of these documents is
incorporated herein by reference.

It has now been found that protein-containing
microorganisms (also referred to herein as "single-cell
proteins") and derivatives thereof, especially
25 homogenized and autolysed derivatives, may be used as
fish and shellfish feed. Particularly effective fish
and shellfish feeds may be produced using the biomass
harvested from a culture medium comprising
methanotrophic bacteria, e.g. biomass produced as
30 described in WO 01/60974, and derivatives thereof.

Viewed from one aspect the invention thus provides
the use of a single-cell protein material or derivative
thereof (e.g. a homogenized or autolysed derivative of a
single-cell protein material) as a fish or shellfish
35 feed or feed additive.

In a further aspect the invention provides the use
of a single-cell protein material or derivative thereof

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(e.g. a homogenized or autolysed derivative of a single-cell protein material) in the manufacture of a fish or shellfish feed, or in the manufacture of a fish or shellfish feed additive.

5 As used herein the term "derivative" when used in relation to a single-cell protein material, e.g. a microbial culture, includes any product which may be derived from such a material using a downstream processing technique or techniques (e.g. a series of
10 techniques) known in the art, such as separation of a single-cell protein material from a fermentation medium or liquid by centrifugation and/or ultrafiltration methods, or by dewatering, etc. Such techniques also include those which may be used to break down the
15 single-cell material (e.g. the bacterial cells), such as homogenization and/or autolysis.

 As used herein, the term "homogenized" when used in relation to a single-cell protein material is intended to define such a material in which a substantial
20 proportion (e.g. substantially all) of the cells are broken down, i.e. disrupted or disintegrated, e.g. as a result of mechanical disruption. Such homogenized materials will generally consist of a viscous protein slurry containing both soluble and particulate cellular
25 components.

 The term "autolysis" as used herein is intended to encompass a process in which endogenous enzymes contained within a cell, such as nucleases and proteases, digest the components of the cell.

30 A preferred aspect of the invention relates to the use of the products herein described as a feed material for shellfish, for example in shellfish cultivation. Examples of shellfish to which the products herein described may be fed include mussels, oysters, scallops,
35 etc.

 Surprisingly, it has been found that the products herein described are particularly effective in improving

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(e.g. increasing the rate of) detoxification or decontamination of shellfish (especially mussels) contaminated by toxic algae. For example, these may be used to detoxify toxic algae-containing shellfish to levels that are non-toxic to human beings.

In a yet further aspect the invention thus provides a method of detoxifying shellfish (e.g. mussels), said method comprising supplying to said fish an effective amount of a single-cell protein material or derivative thereof as herein described.

The product herein described may also be used in feeding marine fish, particularly white fish such as cod. For example, this may find use as a feed for fish which are bred for human consumption and may therefore be used in fish farming.

Other fish which may be fed with the material herein described are those kept in aquariums. Such fish include those kept for exhibition or as pets for pleasure, e.g. goldfish, koi carp, tropical fish, etc.

Preferred single-cell protein materials for use in the invention are homogenized single-cell protein materials such as those described in PCT/GB01/00628 (WO 01/60974), the entire contents of which are incorporated herein by reference. However, the basic single-cell protein material described in this reference (i.e. that which has not been subjected to homogenization) may also be used. In this regard, although the material produced in the fermentor may be directly used as the feed material (or feed additive) without further processing, this will generally be subjected to additional downstream processing steps, such as for example centrifugation and/or filtration (e.g. ultrafiltration) processes. Both homogenized and non-homogenized materials will typically be subjected to dewatering (e.g. spray drying) and optionally sterilization prior to use as a feed material or feed additive. A dried homogenizate of the bacterial biomass

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described in WO 01/60974 is a particularly preferred material for use in accordance with the present invention.

5 Single-cell protein materials, e.g. bacterial cultures, which have been subjected to autolysis to break down the cells are also suitable for use in the invention. Such a treatment and the resulting autolysates are described, for example, in British Patent Application No. 0203307.4 filed 12 February 2002,
10 the content of which is incorporated herein by reference (copy attached hereto). The autolysate, more especially the dried autolysate, described in this earlier application is considered particularly suitable for the preparation of feed materials in accordance with the
15 invention.

Particularly preferably, the single-cell protein material for use in the invention will be derived from a microbial culture which comprises at least one methanotrophic bacteria optionally in combination with
20 one or more species of heterotrophic bacteria, especially preferably a combination of methanotrophic and heterotrophic bacteria. Preferably these will be grown in the same culture medium, e.g. using a loop reactor provided with methane, oxygen, ammonia and
25 mineral feeds. Such materials and processes for their preparation are described in PCT/GB01/00628 (WO 01/60974) the entire contents of which are incorporated herein by reference. One particularly suitable combination of bacteria for generating a
30 biomass for use in the invention is *Methylococcus capsulatus* (Bath) (strain NCIMB 11132), in combination with *Ralstonia sp.* DB3 (strain NCIMB 13287) and *Brevibacillus agri* DB5 (strain NCIMB 13289), optionally together with *Aneurinibacillus sp.* DB4 (strain NCIMB
35 13288).

The bacterial culture used to produce the biomass is preferably at least 50%, more preferably at least

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60%, especially at least 70%, in particular at least 75%, e.g. 75 to 95%, more particularly 75 to 80%, by weight methanotrophic bacteria (relative to the total bacterial weight).

5 As used herein, the term "methanotrophic" encompasses any bacterium which utilizes methane, methanol or formaldehyde for growth. The term "heterotrophic" is used for bacteria that utilize organic substrates other than methane, methanol or
10 formaldehyde for growth.

 The bacterial biomass for use in the invention may be formed by growth of the bacteria on a suitable medium or substrate. The exact nature of the growth medium used to produce the biomass is not critical and a
15 variety of suitable substrates may be used. Conveniently, the biomass may be produced by a fermentation process in which oxygen and a suitable substrate such as a liquid or gaseous hydrocarbon, an alcohol or carbohydrate, e.g. methane, methanol or
20 natural gas, together with a nutrient mineral solution are fed to a tubular reactor containing the microorganisms. Such a process is described, for example, in WO 01/60974.

 A particularly preferred biomass material for use
25 in the present invention is that derived from fermentation on hydrocarbon fractions, particularly preferably that derived from fermentation on natural gas.

 For use as a fish or shellfish feed, appropriate
30 amounts of the material herein described will depend on several factors, such as the species of fish or shellfish for which the product is intended. However, such amounts can readily be determined by those skilled in the art.

35 For most applications, especially for feeding shellfish, the feed will be supplied in particulate form (e.g. as granules or in powder form). For feeding

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shellfish, the particles will typically be added to seawater which is then circulated through the holding tanks. Suitable particle sizes may range from 0.5 to 20 μm . However, feeds in the form of a liquid (e.g. as an aqueous slurry) may also be used and are considered suitable for use in feeding shellfish, especially shellfish which are attached to a culture rope.

In a still further aspect the invention thus provides a liquid feed for shellfish (e.g. mussels), said feed comprising an aqueous slurry of a single-cell protein or derivative thereof as herein described.

The invention will now be described in more detail by way of the following non-limiting Examples:

Example 1 - Biomass Extracts

Methanotrophic and heterotrophic bacteria (*Methylococcus capsulatus* (Bath) (strain NCIMB 11132), *Ralstonia* sp. DB3 (strain NCIMB 13287), *Brevibacillus agri* DB5 (strain NCIMB 13289), and *Aneurinibacillus* sp. DB4 (strain NCIMB 13288)) are cultivated as described in WO 01/60974 and the resulting biomass harvested and treated as described in WO 01/60974 to produce a spray-dried, particulate homogenizate, and as described in British Patent Application No. 0203307.4 to produce a spray dried autolysate.

Example 2 - Feeding Shellfish

The homogenizate produced in Example 1 is suspended in sea water at a concentration of from 10 to 1,000 ppm. This suspension of feed is then circulated through a tank containing shellfish (e.g. mussels, oysters or scallops) for a period of from 1 to 3 weeks.

The same feeding regimen may be carried out utilising the autolysate material of Example 1.

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Claims:

1. Use of a single-cell protein material or derivative thereof (e.g. a homogenized or autolysed derivative of a single-cell protein material) as a fish or shellfish feed or feed additive.
2. Use of a single-cell protein material or derivative thereof (e.g. a homogenized or autolysed derivative of a single-cell protein material) in the manufacture of a fish or shellfish feed, or in the manufacture of a fish or shellfish feed additive.
3. A method of detoxifying shellfish (e.g. mussels), said method comprising supplying to said fish an effective amount of a single-cell protein material or derivative thereof (e.g. a homogenized or autolysed derivative of a single-cell protein material).
4. A use or method as claimed in any one of claims 1 to 3, wherein said single-cell protein material is derived from the biomass of a culture of bacteria including methanotrophic bacteria, and optionally at least one species of heterotrophic bacteria.
5. A use or method as claimed in claim 4, wherein said culture comprises *Methylococcus capsulatus* (Bath) (strain NCIMB 11132), *Ralstonia sp.* DB3 (strain NCIMB 13287) and *Brevibacillus agri* DB5 (strain NCIMB 13289), optionally in combination with *Aneurinibacillus sp.* DB4 (strain NCIMB 13288).
6. A use or method as claimed in claim 4 or claim 5, wherein said culture is produced by fermentation on hydrocarbon fractions or on natural gas, preferably from fermentation on natural gas.

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7. A liquid feed for shellfish (e.g. mussels), said feed comprising an aqueous slurry of a single-cell protein or derivative thereof (e.g. a homogenized or autolysed derivative of a single-cell protein material).
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8. A liquid feed as claimed in claim 7 wherein said single-cell protein material is as defined in any one of claims 4 to 6.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A23K1/16 A23K1/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, CAB Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	J.A. NELL: "Comparison of some single cell proteins in the diet of the Sydney rock oyster (Saccostrea commercialis)" PROGRESSIVE FISH-CULTURIST, vol. 47, no. 2, 1985, pages 110-113, XP001126929 WASHINGTON, DC, US ISSN: 0033-0779 the whole document	1,2,4

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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- *E* earlier document but published on or after the international filing date
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International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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